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SHORT COMMUNICATION

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Tetraploid European paeonies (*Paeonia*) show a homogeneous karyotype asymmetry and structure

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Abstract

Within a practical course of cytotaxonomy organized in Pisa (Italy) on February 2024 by the Group for Floristics, Systematics and Evolution of the Italian Botanical Society, we tested whether relevant differences in karyotype asymmetry and structure occur in four tetraploid European taxa from *Paeonia* sect. *Paeonia* (*P. mascula* subsp. *russoi*, *P. officinalis* subsp. *huthii*, *P. officinalis* subsp. *italica*, and *P. peregrina*). Our results point towards a homogeneous karyotype asymmetry and structure among studied tetraploid species, with no statistically significant difference among taxa and high overlap in variation highlighted by PCA.

Keywords

Cytogenetics, cytostematics, cytotaxonomy

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Introduction

The Boreal genus *Paeonia* is the only member of the family Paeoniaceae (Saxifragales) and includes ca. 40 species (Tamura 2007). It consists of three monophyletic sections: *P.* sect. *Paeonia* (which includes most herbaceous species, from Eurasia), *P.* sect. *Moutan* DC. (including woody species endemic to western China), and *P.* sect. *Oanepia* Lindl. (including herbaceous species from North America) (Sang et al. 1997). The basic chromosome number of this genus is $x = 5$, which is most likely ancestral (Carta et al. 2020). Three ploidy levels have been documented: diploids ($2n = 10$, found in all sections), tetraploids ($2n = 20$, found only in *P.* sect. *Paeonia*) and (mostly artificial) hybrid triploids with $2n = 15$ chromosomes (Hong et al. 1988; Yang et al. 2017).

Due to the large chromosomes (10–15 μm long; Okada and Tamura 1979) as compared to other angiosperms (Badr and El-Shazly 2021), this genus is an ideal model to practice with karyomorphology, which is a simple, inexpensive and powerful approach to obtain useful basic comparative information in systematic studies (Astuti et al. 2017). This approach typically involves measuring chromosomes (i.e. length of long arm [L], short arm [S]) in spread metaphase plates to describe the phenotypic aspect of a chromosome complement (Levin 2002; Guerra 2012).

Metaphase plates of selected *Paeonia* taxa were utilized during a practical course on cytotaxonomy organized in Pisa (Italy) between February 6 and 9, 2024 by the Group for Floristics, Systematics and Evolution of the Italian Botanical Society. The main objective was to test whether relevant differences in karyotype asymmetry and structure could be identified in selected tetraploid European taxa from *P.* sect. *Paeonia*. Commonly used traits for this analysis included total haploid (monoploid) chromosome length (THL), the mean centromeric asymmetry (M_{CA}), the coefficient of variation of chromosome length (CV_{CL}), and the coefficient of variation of centromeric index (CV_{CI}) (Peruzzi and Altınordu 2014).

Materials and methods

One of the authors (LP) prepared a set of permanent microscope slides about twenty years ago (between 2002 and 2005), as part of a study on Italian *Paeonia* species, whose results were only partially published concerning the sole chromosome numbers (L. Peruzzi in Passalacqua and Bernardo 2004).

Squash preparations were made on root tips obtained from plants temporarily cultivated in pots at the Botanical Garden of the Calabria University, Arcavacata di Rende, Cosenza (Table 1). Root tips were pre-treated with 0.4% colchicine for 3 hours and then fixed in Carnoy solution for 1 hour. After hydrolysis in 1N HCl at 60 °C for 7.5 minutes, the tips were stained with leuco-basic fuchsine.

A total of 14 metaphase plates (Table 1) were analyzed by the course participants using MATO software (Altınordu et al. 2016; Liu et al. 2023). The focus was on the following quantitative traits:

- THL (total haploid [monoploid] length of chromosome complement): a proxy of genome size (Carta and Peruzzi 2016; Franzoni et al. 2024) calculated as the sum of the length of all the chromosomes in a metaphase plate, divided by the ploidy level (Peruzzi and Altınordu 2014);
- M_{CA} (mean centromeric asymmetry): intrachromosomal karyotype asymmetry (Peruzzi and Eroğlu 2013) calculated as the mean value of the difference between the two (complementary) proportions L/(L+S) and S/(L+S), multiplied by 100;
- CV_{CL} (coefficient of variation of chromosome length): interchromosomal karyotype asymmetry (Paszko 2006) calculated as the standard deviation of chromosome lengths (L+S) in a complement, divided by the mean chromosome length and multiplied by 100;
- CV_{CI} (coefficient of variation of centromeric index): degree of heterogeneity in the position of centromere in a karyotype (Zuo and Yuan 2011) calculated as the standard deviation of centromeric index S/(L+S) in a complement, divided by the mean centromeric index and multiplied by 100 (Paszko 2006).

To test for differences among taxa, univariate (non-parametric Mann-Whitney pairwise test) and multivariate (PCA, after logarithmic standardization) statistics have been conducted in PAST 4.17 (Hammer et al. 2001; Hammer 2024).

Results

All taxa studied had $2n = 4x = 20$ chromosomes. The variation of each karyomorphological trait is presented in Table 2.

According to univariate statistics, no significant differences were observed among the four studied taxa, for any of the considered karyomorphological parameters. The PCA (Fig. 1), whose first two components account for 85% of variation, show overlap among groups, although higher CV_{CI} and CV_{CL} values slightly separated the two accessions of *P. mascula* subsp. *russoi* from the others.

Table 1. Source of the tetraploid ($2n = 4x = 20$) *Paeonia* taxa studied, and the number of studied metaphase plate microphotographs.

	Locality, date, and collector(s)	**metaphase plates (*N*)**
Paeonia mascula (L.) Mill. subsp. *russoi* (Biv.) Cullen & Heywood	Italy, Sicily, Iblei (Valle Cava Grande), 10 July 2003, *L. Peruzzi et N.G. Passalacqua*	2
Paeonia officinalis L. subsp. *huthii* Soldano	Italy, Liguria (Monte Toraggio), 19 June 1995, *L. Bernardo et N.G. Passalacqua*	4
Paeonia officinalis L. subsp. *italica* N.G.Passal. & Bernardo	Italy, Abruzzo, Gran Sasso (Prati di Tivo), 13 July 2001, *L. Bernardo et N.G. Passalacqua*	5
Paeonia peregrina Mill.	Bulgaria, 1 August 2001, *D. Uzunov*	3

Table 2. Karyomorphological traits calculated by the participants for selected tetraploid European Paeonia taxa.

Metaphase plate	$2n$	x	THL (µm)	M_{CA}	CV_{CL}	CV_{CI}
Paeonia mascula subsp. *russoi* 1	20	5	112.38	35.02	19.26	23.26
Paeonia mascula subsp. *russoi* 2	20	5	116.63	32.44	24.54	23.04
Paeonia officinalis subsp. *huthii* 1	20	5	116.58	28.40	25.02	27.30
Paeonia officinalis subsp. *huthii* 2	20	5	103.27	22.43	14.06	20.06
Paeonia officinalis subsp. *huthii* 3	20	5	126.55	17.37	18.85	20.89
Paeonia officinalis subsp. *huthii* 4	20	5	103.73	26.71	14.79	23.87
Paeonia officinalis subsp. *italica* 1	20	5	126.36	23.12	17.24	27.39
Paeonia officinalis subsp. *italica* 2	20	5	101.14	24.55	21.25	22.24
Paeonia officinalis subsp. *italica* 3	20	5	114.59	22.18	22.34	20.18
Paeonia officinalis subsp. *italica* 4	20	5	117.54	26.35	15.01	21.22
Paeonia officinalis subsp. *italica* 5	20	5	93.41	32.02	21.47	24.04
Paeonia peregrina 1	20	5	106.36	28.25	16.14	26.01
Paeonia peregrina 2	20	5	100.33	30.50	21.29	21.74
Paeonia peregrina 3	20	5	122.11	29.48	18.06	25.27

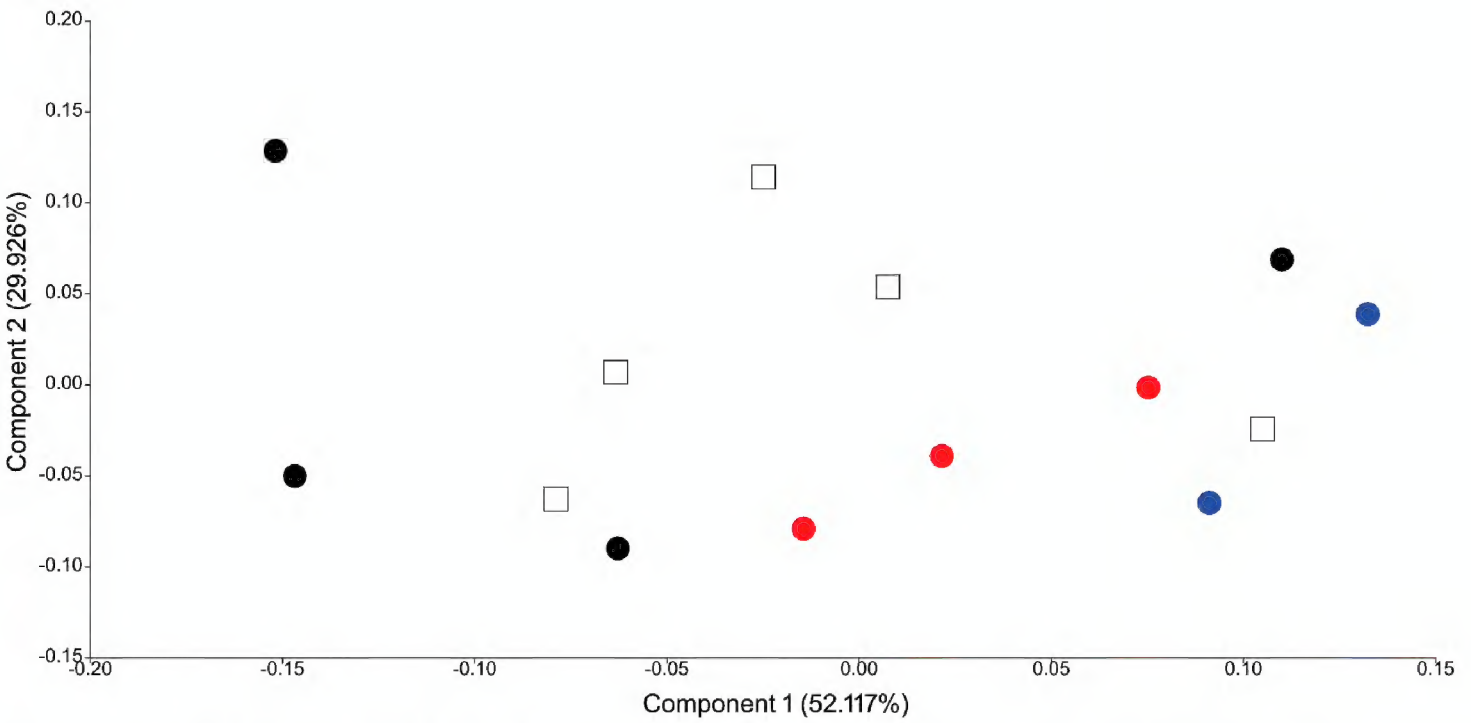

Figure 1. PCA illustrating the overall variation of karyomorphological traits in the four studied tetraploid *Paeonia* taxa. Blue dots: *P. mascula* subsp. *russoi*; black dots: *P. officinalis* subsp. *huthii*, black empty squares: *P. officinalis* subsp. *italica*; Red dots: *P. peregrina*.

Discussion

The chromosome number for Italian *P. mascula* subsp. *russoi*, *P. officinalis* subsp. *huthii*, and *P. officinalis* subsp. *italica* were already reported in Passalacqua and Bernardo (2004), while the count for *P. peregrina* from Bulgaria was not published before, but agrees with data published by Koeva and Sarkova (1997).

Regarding karyomorphological traits, our results suggest homogeneous karyotype asymmetry and structure among the studied tetraploid *Paeonia* species, with no statistically

significant differences between taxa and high overlap in variation highlighted by PCA. Despite the very limited sampling, which prevents from any conclusion, the only species somehow distinct is *P. mascula*, which is also taxonomically more distinct with respect to *P. officinalis*/*P. peregrina* (Passalacqua and Bernardo 2004), based on leaf and root structure.

To our knowledge, this is the first quantitative study on this topic. Our findings are consistent with previous qualitative karyological observations of tetraploid *Paeonia* taxa from Europe (Tzanoudakis 1983; Schwarzacher-Robinson 1986; Punina 2005). This homogeneity seems even more extensive and possibly applying to large portions of *P.* sect. *Paeonia*. Indeed, for instance, CV_{CL} and CV_{CI} values calculated in the studied taxa are very similar to those reported for the tetraploid *P. mairei* H.Lév., endemic to China (Chen et al. 2023).

The similarity in karyotype structure in such tetraploids can hinder the possibility to highlight their allotetraploid status (see, e.g., Sang et al. 2004; Punina et al. 2021) using traditional Feulgen approaches. However, molecular cytogenetic techniques like GISH have been effective in cultivated hybrid varieties (Cui et al. 2022), and could be valuable in studying wild species.

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